"BIO-ASSISTED SYNTHESIS OF METAL OXIDE NANOMATERIALS AND THEIR APPLICATIONS IN DENTISTRY"

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By

Dr. SHUBHA P.

Research Scholar
Department of Studies in Materials Science
Mangalagangotri, Mangalore University

Under the Guidance of

PROF. K. BYRAPPA

Pro-Vice Chancellor and Dean Research
Adichunchanagiri University
B.G. Nagara, Mandya District, Karnataka, India
Former Vice Chancellor, Mangalore University

DEPARTMENT OF STUDIES IN MATERIALS SCIENCE
MANGALAGANGOTRI, MANGALORE UNIVERSITY
KONAJE, DAKSHINA KANNADA, KARNATAKA, INDIA

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SYNOPSIS

1. Introduction

In the current era of advanced functional materials, the nanotechnology and nanomaterials holds a salient position in various fields like device development, medicine and dentistry, food and agriculture, aviation, etc. Nanotechnology has become an extremely active field of research in the last decade, because of its potential application in different areas of medicine like pharmacy, tissue engineering, and dentistry. The unique properties of nanosized materials, which are the subject of quantum mechanical principles has attracted the researchers across the world towards the development of nanotechnology-based materials. Due to their exceptionally tiny size, functionalization capacity, and enhanced activity, the nanomaterials are largely being explored for biological and biomedical applications among which the dental and oral care applications are also a prime area of research. Nanotechnology has emerged as a promising area of interest in dentistry due to variety of new treatment options it offers. The main purpose of using nanotechnology in dentistry is achieving better mechanical properties, higher abrasion resistance, less shrinkage, and improved optical and aesthetic properties and antibacterial properties etc. Interest in the use of nanomaterials based dental products/devices in clinical dentistry is growing.[1] Nanotechnology has been used in the production of a wider range of dental materials such as light polymerizable composites and their bonding systems, impression materials, ceramics, dental implant coatings, bioceramics, etc.[2] Rising attention towards application of nanotechnology in dentistry is leading to the emergence of a new field called nano-dentistry.[3] Among several classes of nanotechnology based dental materials, metal oxides have been one of the key areas of interest for dental researchers. Metallic materials have always been an integral part of dentistry since the time immemorial. Oxides of Zn, Ti, Al, Zr, Se, Cu, Ag and Sr have found various applications in clinical dentistry till today. The nanometal oxides are extensively explored for their application in dentistry from antibacterial dental cements to fillers in various prosthodontic materials to enhance the optical, physical and mechanical

properties.[4] Each year, thousands of tons of metallic nanoparticles (NPs) are produced and utilized in various fields including dentistry.

Despite the major leap that the nanotechnology has taken towards the biomedical applications in recent years, the biocompatibility issue is always a major area of concern for nanotechnologists across the world. When scope of the nanomaterials for biomedical applications is considered, issues like bioactivity, biocompatibility, toxicity and interfacial properties between bio and nanomaterials will come into picture. When the biomedical applications of nanomaterials are considered, conventional Materials Science and Engineering faces challenge in the synthesis as well as microstructural development, as the requisite for general nanomaterials need to be focused on exceptional medical requirements.[5] Most of the industrially scaled up metallic NPs for biomedical applications are being synthesized under extreme experimental conditions that are hazardous to health and environment. In this regard, synthesis of metallic NPs using biological or bio-assisted synthesis methods is gaining popularity. Organic compounds, proteins, plant active biomolecules and microbes are used in the reduction of metallic salts to their respective nanometallic forms having improved biological activity and lesser toxicity. The bio-assisted synthesis methods are believed to have lesser toxic effects on health and environment as well as to have tailor made properties to suitably adapt them for different applications. Preparation of nanomaterials with the use of biomolecules often has excellent control on the morphology and size of the final products.[6]

There are two types of bio-assisted synthesis methods of NPs. The first method involves the use of plant active principles which act as reducing, capping and stabilizing agents in nanomaterials synthesis. Other method involves the use of microorganisms like bacteria, yeast and fungi.

Among the bio-assisted synthesis methods, plant extract mediated synthesis is very popular, especially in the fabrication of nanometal oxides, due to the fact that plant based biological molecules undergo a highly controlled assembly which makes them suitable as biological agents to synthesize these kinds of nanomaterials. Methods involving the use of plant extracts as bioreductants is usually simple, inexpensive, involves single step in general and environmentally friendly.[7] Besides being environmentally benign, the bio-assisted synthesis can aid in the production of large

quantity of metallic NPs which are devoid of contamination with desired size and shape.[8]

Considering this, in the present research work, the candidate has synthesized two important nanometal oxides used in dentistry, ZnO and TiO_2 using bio-assisted synthesis methods by means of plant extracts having medicinal importance. The candidate has employed either of co-precipitation, bio-hydrothermal or microwave as energy source in synthesizing ZnO and TiO_2 NPs and their metal doped variants. The dopant metals used are Cu and Ag as they have high significance in biomedical industry. Aqueous extracts of *Emblica offcinalis* fruit, *Punica granatum* peel *Piper betel* leaves and hydroalcoholic extract of *Ocimum sanctum* were bioreductants used in the present study. Synthesized ZnO and TiO_2 NPs and their doped variants were investigated for their real-time applicability in various fields of dentistry.

2. Need of the Study

As mentioned in Section 1, metal oxides are one of the key components of several dental materials. They have found their applications in almost all the branches of dentistry. Several metal oxides that are being used in dentistry are of bulk size having no indigenous antibacterial, antioxidant and anti-inflammatory properties. Some of the metal oxides that are used in restorative dentistry and prosthodontics do not provide the required amount of mechanical support for the restorations as well as therapeutic efficiency in terms of antimicrobial action. With plethora of dental materials and products available, the dental diseases should have been on hold, but the scenario is not so. There is still need of dental materials and products with improved antibacterial activity, improved physical and mechanical properties, less microleakage around the margins of restorations, good osseointegration around the implant surfaces and so on. Metal and metal oxide nanomaterials which are synthesized using biological molecules are known to have controlled size and morphology, biocompatibility, stability etc, and incorporation of such nanomaterials into dental products may improve their antibacterial, physico-mechanical property and clinical performance.

Considering this gap, in the present research work, the candidate has explored the possibility of using ZnO and TiO_2 NPs that are synthesized using bio-assisted methods to improve the quality of existing dental materials.

3. Objectives

- Bio-assisted synthesis, doping and characterization of ZnO and TiO_2 nanomaterials using multi-energy methods utilizing aqueous/hydro-alcoholic phytoextracts as bioreductants.
- Analysis of *in vitro* biological activity and toxicity of synthesized nanomaterials using antibacterial, hemolysis and WST-8 (cell viability) assays.
- To study the effect of incorporation of nanomaterials synthesized using bio-assisted methods on biological and physicomechanical properties of few dental materials based on their size, morphology and doping.
- Formulation of dentifrices (toothpaste and mouthwash) using nanomaterials synthesized using bio-assisted methods combined with selected herbal extracts and assaying their antibacterial efficiency on selected dental pathogens.
- *In vitro* toxicity assessment of nano-herbal dentifrices against balb 3T3 mice fibroblast cells as well as assessment of the effect of nano-herbal dentifrices on surface properties of extracted natural teeth.

4. Review of Literature

A detailed review of literature on bio-assisted synthesis, characterization, *in vitro* and *in vivo* toxicity of ZnO and TiO_2 NPs and current status on application of ZnO and TiO_2 NPs in dentistry was carried out.

ZnO and TiO_2 are the two metal oxides which are frequently looked for their biomedical applications. In general it has been observed that there is always a need to synthesize uniformly sized, biocompatible and biologically active nanomaterials in a scalable manner for several biomedical applications. In this regard, several researchers across the world have used plant active biomolecules present in several plant extracts in bio-assisted synthesis of ZnO and TiO_2 NPs and their doped variants. Most of these studies have focused on synthesizing NPs having enhanced biological activity, reduced toxicity and scalability. Several research groups have established that plant active biomolecules are able to produce NPs having controlled size, morphology, enhanced biological activity and reduced toxicity. These studies have stressed up on the importance of optimizing the experimental parameters to control the size, morphology, biological activity and toxicity of NPs which includes temperature, pressure, *pH*, method of synthesis, concentration of plant extract, concentration of active biomolecules (phenolics, flavonoids, proteins etc) in the

selected plant extract, concentration of dopant metal (in case of metal doping) etc. It has been observed that among several synthesis methods, hydrothermal/solvothermal technique combined with phyto-assisted synthesis yields uniformly sized and bioactive ZnO and TiO_2 NPs, most of which are suitable for biomedical applications. In recent years several researchers have adapted benign synthesis methods during hydrothermal experiments. Temperature of reactant mixture can be as low as 100 to 200°C under autogenous pressure. People have found that high phenolic and flavonoid contents in selected plant materials gives better yield of NPs irrespective of method of synthesis adapted.[9-17] Another popular method that is being explored is bio-assisted synthesis of ZnO and TiO_2 NPs sourced with microwave energy. Microwave-assisted method of synthesis is rapid, facile and uses clean energy source, hence produces less burden on environment. Bio-assisted synthesis sourced by microwave energy lead to formation of ZnO and TiO_2 NPs having smaller size and spherical shape.[18-20]

Doping of metal oxides leads to the formation of smaller sized NPs with higher reactivity. In a significant study, Zare *et al* (2019) used the leaf extract of *T vulgaris* for the first time to synthesize Ag doped ZnO nanocomposites using bio-hydrothermal approach. The *T vulgaris* leaf extract has served as both reducing and capping agent that play a critical role in production of ZnO-Ag nanocomposites. The *in vitro* antimicrobial activity of nanocomposites displayed high antimicrobial potency on selective gram negative and positive food borne pathogens.[21]

The major hindrance that the scientific community is facing in the biomedical application of NPs is their possible potential toxicity towards various tissues and organs. It has been proved in several instances earlier that the toxic chemical reducing agents that are used in the synthesis of NPs are a major burden to human health and environment. Considering this, several research groups are now attracted towards benign bio-assisted synthesis methods to synthesize NPs for several applications. When it comes to the biomedical applications, the bio-safety is the prime area of concern when developing NPs based biomaterials. In this regard, several investigators earlier have compared the *in vitro* and *in vivo* toxicity of ZnO and TiO_2 NPs synthesized by chemical and bio-assisted methods and have found that bio-assisted methods have produced NPs having lesser toxicity and their toxicity is majorly concentration dependent. Hence optimizing the concentration would help to produce nanomaterials which are biocompatible and have bio-safety.

In bio-assisted synthesis of metal oxide NPs, plant extracts act as capping and stabilizing agents controlling size and morphology of NPs. Capping agents stabilize NPs through different mechanisms which include electrostatic stabilization, steric stabilization, stabilization by hydration forces, and stabilization using Van-der Waals forces. Stabilization of NPs is significant for its application and also determines toxicity of NPs.[22] The debate on toxicity of ZnO NPs still exists for various reasons, as some investigators have found material to be biocompatible and non-toxic while some studies have reported toxicity on mammalian cells both in *in vitro* and *in vivo* conditions.

The size and surface morphology of NPs play a significant role in determining the NP toxicity. At the same time, the type and metabolism of the cells against which the NPs are targeted will also determine the toxic effect of NPs.[23] In a significant study, Zhou *et al* (2015) investigated the toxicity of 17 different ZnO NPs having different sizes and surface chemistry on embryonic zebra fish, where the exposure concentrations ranged from 0.016 to 250 mg/lt. ZnO NPs with chemical modifier based modification, regardless of the type, resulted in mortality at 24 hr post-fertilization (hpf) while unmodified particles did not induce significant mortality until 120 hpf. This is a clear evidence of toxicity due to chemical surface modifications.[24] Earlier, in several occasions, it has been found that ZnO NPs synthesized by bio-assisted methods show better antimicrobial activity, anti-inflammatory and antiproliferative activity compared to chemically synthesized variants and are less toxic to cells and tissues both in *in vitro* and *in vivo* conditions.[25-39]

Similarly, the *in vitro* and *in vivo* toxicity of TiO_2 NPs has also been studied extensively**.** TiO_2 NPs may enter the systemic organs from lungs and gastrointestinal tract, but the rate of translocation seems to be low. The oral exposure occurs mainly through the food products and a little through ingestion of toothpaste (in children below 6 years). The entry of TiO_2 into viscera might induce pathological lesions of liver, spleen, kidneys and brain, which increases with increase in concentration of TiO_2 NPs. Long term exposure studies on rats have shown that these NPs are reported to cause lung tumors.[40] Several researchers have found that there exists an intrinsic toxic nature in most of the chemically synthesized TiO_2 NPs, immaterial of the crystallographic form.[41-44] Hence in recent years, synthesis of TiO_2 NPs is shifting towards bio-assisted methods. The TiO_2 NPs that are synthesized by bio-assisted methods have shown better biological activity including anticancer activity.[45-47]

Currently, both ZnO and TiO_2 NPs have found several biomedical applications. Both these materials are being explored for their possible applications in targeted drug delivery/gene therapy and anticancer therapy, antimicrobial therapy and generation of smart wound dressings, osteogenic and angiogenic materials in tissue engineering for advanced surgical reconstructions and rehabilitations, coating on surface of implants.

ZnO is one of the most commonly used metal oxides in dentistry; hence it is a material of choice in research towards developing NPs based dental materials. ZnO NPs have been investigated for their applicability as fillers in dental composites, sealer cement in endodontics, antibacterial agent against dental pathogens, mainly the caries causing microbes, as a filler to improve the mechanical properties of dental adhesive materials, etc.[48-56]

The Titanium metal has always been a material of choice in preparation of dental implant surfaces as a core material or implant surface coating. The nano oxide form of Ti, the nano TiO_2 has been greatly explored for its applicability as osteogenic material in maxillofacial surgical procedures, in Guided Tissue Regeneration (GTR) and Guided Bone Regeneration (GBR) techniques.[57] Apart from their applications in maxillofacial and periodontal applications, the TiO_2 NPs have also been looked as potential materials to enhance the mechanical and antibacterial property of dental cements, composites materials and denture base materials.[58-60]

Based on the variety of their applications, there is a huge demand for these materials in biomedical industry. The prime factor in selecting any biomaterial for human application is their bio-safety. Several researchers have reviewed the presently available chemically synthesized ZnO and TiO_2 materials (both bulk and nanoform) and are of the opinion that there are different levels of toxicity exhibited by these materials ranging from allergies to pneumotoxicity and carcinogenicity. These toxic changes are majorly attributed to the toxic chemical and other harsh experimental conditions that are followed during their synthesis. To address the problem of toxicity and improve their biological activity several research groups are working towards developing environmentally friendly and non-toxic ZnO and TiO_2 NPs using bio-assisted methods. The plant active biomolecules have the ability to chelate the metals from their respective salts. Several factors that are considered in selecting these plant biomolecules for their role as bioreductants and capping agents in NPs

synthesis are discussed above. It is of a general opinion that the metal oxides (including the nanoforms) that are synthesized using phyto-assisted or bio-assisted methods show better antioxidant, antibacterial, antifungal, anti-inflammatory and anticancer activities compared to their chemically synthesized counterparts. The metal oxide NPs that are synthesized using these bio-assisted methods usually yield uniform sized NPs whose shape can be customized by choosing appropriate type of biomolecules. It is observed that only a very high concentration of biologically synthesized NPs cause mild to moderate toxicity in several animal models. Due to these unique advantages, the ZnO and TiO_2 NPs that are synthesized by these bio-assisted methods are highly being investigated for their applications in medicine and dentistry. Several significant studies that are mentioned above have used these biosynthesized ZnO and TiO_2 NPs in developing materials for targeted drug delivery and cancer therapy, antimicrobial agents, anticancer agents, tissue engineering materials in orthopedics and maxillofacial surgery, smart wound dressing materials etc. Several types of dental formulations are developed using ZnO and TiO_2 NPs for maxillofacial, restorative, endodontic and prosthetic applications.

In the current research work, ZnO and TiO_2 NPs that are synthesized using bio-assisted methods are investigated for their applicability as antimicrobial, antiproliferative agents, filler to improve the mechanical properties of dental cements and PMMA denture base material, photoinitiator material for photodynamic therapy. Apart from these applications, novelty of this research work also lies with the development of nano-herbal toothpaste and mouthwash formulations using synthesized NPs mixed with selected plant extracts to achieve enhanced therapeutic activity.

5. Methodology

- ZnO and TiO_2 NPs, Cu doped ZnO and TiO_2 NPs and Ag doped ZnO NPs were synthesized using bio-assisted multienergy methods using co-precipitation, bio-hydrothermal and microwave- assisted synthesis methods. For bioreduction of respective metal salts, aqueous extract of *Piper betel* leaves, *Punica granatum* peel, gallic acid isolated from *Emblica officinalis* fruit and hydroalcoholic extract of *Ocimum sanctum* were used. Soxhalet extraction method was used in the

preparation of all bioreductants except *O sanctum* hydroalcoholic extract, which was prepared by cold maceration method.

- Physical and morphological characterization of synthesized NPs was carried out using powder XRD, FTIR, Uv-Vis spectroscopy, SEM/FESEM, EDS and DLS.
- Antimicrobial activity of synthesized NPs and their doped variants was carried out against pathogenic bacteria, the *S mutans*, *L acidophilus*, *S aureus* using well-diffusion, disk diffusion and colony counting techniques.
- *In vitro* hemolytic activity of synthesized NPs was assayed against chick blood using Halfman's group standard procedure. Possible cytotoxicity of ZnO and TiO_2 NPs and their doped variants was assayed against Balb 3T3 mice fibroblast cell lines using WST-8 assay using CCK-8 kit.
- ZnO and Ag doped ZnO NPs synthesized using microwave assisted method using *P granatum* peel was incorporated into ZnO cement powder that is used in clinical dentistry to prepare ZOE cement. The prepared powder was mixed with eugenol liquid and checked for change in setting time and antimicrobial activity against *S mutans* and *L acidophilus.* The compressive strength of set ZOE cement post incorporation of ZnO/ZnO Ag NPs was also studied.
- ZnO NPs and Cu doped ZnO NPs synthesized using hydroalcoholic extract of *O sanctum* was incorporated to the powder part of heat cure acrylic PMMA denture base resin in different w/w ratio. The NPs incorporated resin base powder was mixed with the monomer liquid as per manufacturer's instructions. The flexural strength of prepared resin samples were measured using 3-point bend test. The prepared resin specimens were also tested for their possible antifungal activity against *Candida albicans.*
- ZnO NPs synthesized using natural honey was coated on degummed *Bombyx mori* silk fibers using *ex situ* method. XRD of coated fibers was obtained to study the possible changes in the crystallinity of ZnO NPs coated fibres. SEM images were taken to check the possible coating and EDS spectrum was obtained to confirm the presence of ZnO NPs on silk fibre surface. Autoclave and Ethylene oxide sterilization was performed on coated and uncoated fibres and tensile strength measurement was made to assay the possible effect of coating and sterilization on silk fibres. The antimicrobial activity of ZnO NPs coated fibres against *S aureus* was assayed for 6 consecutive days.

- ZnO NPs synthesized using gallic acid isolated from *E officinalis* was also used in formulating nanogels for photodynamic therapy to combat periodontal infection caused by *P gingivalis.* The drug release study was performed using UV-vis spectroscopy. RT-PCR technique was followed to study the effect of nanogel formulations containing ZnO NPs on *P gingivalis* inhibition.
- ZnO NPs synthesized using gallic acid isolate from *E officinalis* fruit was mixed with *O sanctum* hydroalcoholic extract to formulate nano-herbal mouthwashes. The antimicrobial activity of mouthwash formulation was assayed against *S mutans.* Appropriate pharmacological and stability studies were also performed.
- Both bio-hydrothermal and microwave synthesized TiO_2 NPs were incorporated into powder part of Type-II GI cement at 50 mg/g and 100 mg/g concentrations. The effect of TiO_2 NPs incorporation on setting time, compressive strength and antimicrobial activity against *S mutans* (after 24 hr and 72 hr) was studied in detail.
- Microwave synthesized TiO_2 NPs was mixed with *P gantum* peel aqueous extract and other excipients and nano-herbal toothpaste formulations were prepared. Prepared toothpaste formulations were assessed for its pharmaceutical properties and antibacterial activity against *S mutans* and *L acidophilus.* Effect of nano-herbal toothpaste formulations on dentinal tubular occlusion was studied using SEM images.
- Statistical methods like one-way ANOVA, two-way ANOVA and paired T-tests were applied wherever required. SPSS software was used for statistical analysis of the results of various experiments of the study.

6. Significant Findings of the Study

ZnO is one of the commonly explored materials for biomedical and dental applications. In the current research work the ZnO NPs and its metal doped variants Cu-ZnO NPs and Ag-ZnO NPs were synthesized using various plant extracts using different energy sources that are mentioned above.

The size of undoped (pure) ZnO NPs synthesized by various bio-assisted routes varied between 81 and 39 nm. Shape of undoped ZnO NPs varied between irregular flakes to spherical. Bio-hydrothermally synthesized ZnO NPs using natural honey and gallic acid isolated from *E officinalis* fruit extract were uniformly sized and spherical in shape. From experiments related to metal doping of ZnO NPs it became evident

that doping decreased the size of NPs; with the increase in concentration of dopant, there was a corresponding decrease in the size of ZnO NPs. While the Cu doped ZnO NPs were short rod shaped, Ag doped ZnO NPs appeared flakes to spherical shaped under SEM.

Antimicrobial activity of undoped (pure) and metal doped ZnO NPs was performed against the bacteria *S mutans, L acidophilus* and *S aureus.* All forms of ZnO NPs synthesized by bio-assisted methods showed antimicrobial activity. Among them, ZnO NPs that were synthesized bio-hydrothermally using gallic acid isolated from *E offcinalis* as a bioreductant showed maximum antimicrobial activity against all the three tested microbes which proves the superiority of bio-hydrothermal synthesis over other forms of synthesis methods when the biological activity of NPs is considered. Antibacterial activity of Ag-ZnO NPs and Cu-ZnO NPs was performed only against *S mutans* which showed that with the increase in dopant concentration there was a corresponding increase in the antimicrobial activity.

Hemolysis assay was used to assess the possible hemolytic effect of ZnO NPs synthesized by bio-assisted methods in comparison to commercially available DZnO (used in clinical dentistry) particles. It was found that in the tested range of concentrations the hemolysis by undoped ZnO NPs remained well within the hematotoxicity limit of 5%. Among all the tested undoped (pure) ZnO nanoforms, gallic acid synthesized ZnO NPs and 1% pomegranate peel aqueous extract synthesized ZnO NPs caused hemolysis of ~3% showing that these materials had higher safety towards the RBCs. Even the metal doped variants caused hemolysis within the acceptable hematotoxicity range of 5%.

WST-8 assay was performed using balb 3T3 mice fibroblast cell lines to assess the possible cytotoxicity due to undoped (pure) and metal doped ZnO NPs. It is observed that at the highest tested concentration, commercially available DZnO caused more than 50% inhibition of growth of fibroblast cell lines. Bio-hydrothermally synthesized ZnO NPs using gallic acid isolate from *E officinalis* showed better growth promoting activity with less than 30% growth inhibition at the highest tested concentration and ~8% inhibition at the lowest tested concentration. Among metal doped variants it was observed that Cu-ZnO NPs showed slightly more toxicity when compared to undoped ZnO as well as Ag- ZnO NPs.

Several types of ZnO NPs modified dental materials were prepared in the current study using the undoped/metal doped ZnO NPs synthesized using bio-assisted methods. These modified dental materials include:

- Nano ZnO/Ag-ZnO incorporated ZOE cements with inherent antimicrobial properties and improved mechanical strength.
- ZnO/Cu-ZnO modified PMMA heat cure acrylic denture base resin material with moderately improved flexural strength.
- ZnO NPs coated degummed silk fibres with improved tensile strength and antibacterial properties for surgical suture applications.
- Novel ZnO based nanogels as antibacterial photosensitizer for photodynamic therapy (PDT) applications.
- Novel ZnO and *O sanctum* hydroalcoholic extract based 'nano-herbal' mouthwashes having anticariogenic properties.

From the results of applications of undoped (pure) and metal doped ZnO NPs in various dental materials it is observed in general that the incorporation of NPs has improved the mechanical properties of existing dental material and also has brought in inherent antimicrobial activity in many of the formulations. Incorporation of nano ZnO and Ag-ZnO NPs has made the commonly used ZOE cement anticariogenic due to its excellent inhibition of *S mutans.* At the same time the compressive strength of the cement has also increased due to the uniform dispersion of undoped/metal doped ZnO NPs. Coating of ZnO NPs on degummed silk fibres improved the tensile properties of silk fibres and brought in the inherent antimicrobial activity to silk fibres against *S aureus*, a commonly encountered pathogen in wound infections. This is a remarkable finding of the present study as silk sutures have caused suture line infections in several occasions and coating with ZnO NPs could be a real problem solver.

Novel ZnO based nanogels were formulated to act as photosensitizer (PS) in photodynamic therapy (PDT). Apart from being PS in PDT, the nanogels also showed remarkable antimicrobial activity against the major periodontal pathogen, the *P gingivalis.* The ZnO nanogels synthesized for PDT work is under the process of patenting. The 'nano-herbal' mouthwashes that were formulated using *O sanctum* hydroalcoholic extract and bio-hydrothermally synthesized (using gallic acid isolate

form *E officinalis*) ZnO NPs caused excellent inhibition of *S mutans* growth. The mouthwash formulations were stable up to 2 months under refrigeration and up to 1 month under room temperature. There was no alteration in enamel surface microstructure when examined under SEM.

Bio-assisted synthesis of TiO_2 NPs was majorly carried out by bio-hydrothermal and microwave assisted methods. Bio-hydrothermal synthesis was carried out using *P betel* aqueous extract which resulted in TiO_2 NPs of anatase phase and average size of 39 nm and monomodal distribution. The NPs were spherical in shape.

TiO_2 NPs were also synthesized using hydroalcoholic extract of *O Sanctum* using microwave as energy source. Average size of TiO_2 NPs synthesized using microwave assisted method was 63 nm. Morphologically, the microwave synthesized TiO_2 NPs were short rod shaped with blunt edges, which is a unique finding of this synthesis method as the nanotubes, fibres and rod shaped TiO_2 NPs are explored for their biomedical applications to a great deal.

The microwave-assisted synthesized TiO_2 NPs showed a slightly better antimicrobial activity against *S mutans* when compared to bio-hydrothermally synthesized TiO_2 NPs. Hemolysis assay showed that in the tested range of concentrations, the TiO_2 NPs synthesized by bio-assisted methods demonstrated toxicity which remained well within the acceptable limit of hemotoxicity for NPs. WST-8 assay was performed using balb 3T3 mice fibroblast cell lines to assess the possible cytotoxicity due to TiO_2 NPs. Commercially available TiO_2 NPs were used for comparison. Results of the WST-8 assay showed that both the commercially available and synthesized (bio-assisted) TiO_2 NPs showed growth promoting activity in the tested range of concentrations.

The TiO_2 NPs that are synthesized in the current work are used in formulating two dental materials. The first one is TiO_2 NPs modified Type-II Glass Ionomer (GI) cement and the second one is nano-herbal toothpaste using microwave-assisted synthesized TiO_2 NPs and aqueous extract of *P granatum* peel.

Both bio-hydrothermal and microwave-assisted synthesized TiO_2 NPs were incorporated into the powder part of Type-II GI cement at 50 mg/g and 100 mg/g concentrations. It was observed that setting time noticeably decreased in formulations

incorporated with 100 mg/g of TiO_2 NPs. This could be attributed to the formation of multiple nucleation centers due to the presence of TiO_2 NPs that might have hastened the process of cement setting. At the same time, the compressive strength of cement formulations incorporated with 100 mg/g of TiO_2 NPs was relatively high when compared to unmodified Type-II GI cement. The antimicrobial activity of TiO_2 NPs incorporated GI cement against *S mutans* was determined using spread plate technique at 24 hr and 72 hr interval. Average number of colonies produced by cement formulations containing 100 mg TiO_2 NPs/g of GI powder was statistically significant with less than 100 colonies after 24 hr of incubation. The results of antimicrobial activity of these two formulations remained consistent even after 72 hr.

Nano-herbal toothpaste formulations are one of the novelties of this thesis work, where microwave-assisted synthesized TiO_2 NPs using *O sanctum* hydroalcoholic extract were combined with 10% aqueous extract of *P grantum* peel to prepare nano-herbal toothpaste formulations with several advantages. Firstly all the toothpaste formulations (except the one incorporated only with NPs) demonstrated an excellent antimicrobial activity against two cariogenic bacteria; the *S mutans* and *L acidophilus.* The formulation which contained only TiO_2 NPs without the incorporation of 10% *P granatum* peel aqueous extract showed activity only against *S mutans.* The antimicrobial activity of toothpaste was mainly dependent on the plant extract concentration since the amount of NPs used in all the formulations remained constant. Presence of *P granatum* has enhanced the spectra of antimicrobial activity and has caused the inhibition of *L acidophilus* too. The cytotoxicity exhibited by these toothpaste formulations against Balb 3T3 mice fibroblast cell lines remained within 10-12% showing that the prepared toothpaste formulations were non-toxic in nature.

A very interesting phenomenon that was noticed in the present study is dentinal tubular occlusion by nano-herbal toothpaste formulations. The exposed dentinal tubules were completely occluded by nano-herbal toothpaste formulations. F1 formulation containing 30 mg of *P granatum* peel extract and 100 mg of TiO_2 NPs caused partial tubular occlusion (Score 3). With the increase in the concentration of *P granatum* there was a corresponding increase in the dentinal tubular occlusion too. This significant result shows that this nano-herbal formulation can be developed into a *de novo* toothpaste formulation having both antimicrobial effects as well as anti dentinal hypersensitivity activity.

7. Future Perspectives

Modern dentistry is in state of transition. Adapting interdisciplinary research approach to develop novel materials and technologies for prevailing issues of dentistry is the need of the day. Apart from the through clinical knowledge and skills, there is a great need of material knowledge too for the practicing dental surgeons, academicians as well as beginners of dental curriculum. In several instances, the field has witnessed many failures in the therapeutic outcome because of pitfalls in the materials selected and used. Many a times the material aspect of dental therapeutics has played a major role in the success or failure of the treatment. Many materials that are being used clinically are not highly biocompatible, but are still being used as there are a very few alternatives. Therefore it is the need of the day to explore novel alternatives to the conventional dental materials to improve the treatment outcome in terms of improved biomechanical properties, antimicrobial activity and improved shelf life.

Metal oxides are one of the key components of several dental materials. They have found their applications in almost all the branchess of dentistry. Several age old metal oxides that are being used in dentistry are of bulk sized having no indigenous antibacterial, antioxidant and anti-inflammatory properties.

Considering this gap, the present research work was taken up by the candidate to explore the possibility of using ZnO and TiO_2 NPs that are synthesized by bio-assisted methods to improve the quality of existing dental materials.

It is observed that both the metal oxides (ZnO and TiO_2 NPs) used in this work are synthesized by bio-assisted methods and has shown some amount of synergistic effect which has brought in the improvement in the material properties. Both the NPs are extensively tested for their biosafety under *in vitro* conditions, which has given favorable results.

Considering this fact, the current thesis work opens up an ample scope for further research in developing nanotechnology based range of dental products having improved physicomechanical and biological properties and lesser toxicity. It is also observed that several custom-made range of products can be developed using materials synthesized in the present work for both dental and other biomedical applications. The present research work has opened up a new pathway for the dental fraternity to elevate the modern dentistry to a newer level using nanomaterial and nanotechnology.

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